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THE STARTLE REFLEX AS A LEARNING TASK: APPARATUS AND TEST PARAMETERS

Darrell G. Evans, Staff Sergeant, USAF
Linda B. Anderson, Senior Airman, USAF
Sandra Z. Brothers, Second Lieutenant, USAF
Stuart L. Hutchinson, Sergeant, USAF
Thomas G. Wheeler, Ph.D.

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USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, TX 78235-5301

NOTICES

This interim technical paper was submitted by personnel of the Vulnerability Assessment Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-05-58.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

Darrell G. Evans

DARRELL G. EVANS, SSGT, USAF
Project Scientist

DONALD N. FARRER, Ph.D.
Supervisor

Jeffrey G. Davis

JEFFREY G. DAVIS, Colonel, USAF, MC
Commander

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The objective of this report is to define the startle reflex inhibition learning task and to present results of a pilot study designed to determine the experimental conditions which produce the most consistent learning curves. The startle response (muscle flinch) to each tone stimulus was recorded via an accelerator voltage output. The three primary task parameters--tone intensity, interstimulus interval (ISI), and adaptation period--were evaluated to determine which parameters were important in producing consistent learning curves. The results indicate that ISI and adaptation period have little effect on the inhibition learning curves and tone intensity proved to be the primary variable for the range of conditions used in this study. Keywords:

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THE STARTLE REFLEX AS A LEARNING TASK: APPARATUS AND TEST PARAMETERS

INTRODUCTION

The research goals of this laboratory are to evaluate the effects of hazardous environments on an animal's ability to perform assigned tasks. This report reflects our efforts to improve the existing behavioral test battery by adding a new learning task. Our learning task, shuttle avoidance, has proven to be an insensitive measure of learning ability when the stressor produces motor deficits. Therefore, we have added a new learning task to the test battery that does not require extensive motor activity. The objective of this report is to define the new task (startle reflex inhibition) and present results of a pilot study designed to determine the experimental conditions which produce the most consistent learning curves. The new task will subsequently be used to evaluate learning deficits produced by exposure to ionizing radiation and chemical warfare agents.

The task consists of presenting repeated startle stimuli (loud tone) and measuring the animal's muscular responses to each stimulus. The rat is placed in a cage mounted on a vertical accelerometer. After a short adaptation period, each subject receives 20 stimulus presentations with a fixed interstimulus interval (ISI) (2). The animal's response (muscle flinch) to each stimulus is recorded as an accelerator maximum peak-to-peak voltage output within 100 ms of the onset of the tone. The series of responses are then compared in terms of magnitude to determine how long (how many stimulus presentations) the animal took to habituate to the stimulus. The term "learning curve" is used to refer to the function generated by comparing response magnitude vs. number of stimulus presentations.

METHODS

A startle reflex apparatus has been constructed similar to that used by Isom and Hammond (3). The apparatus is described in detail in the Appendix. In summary, the apparatus consists of three integrated parts: a stimulus generator with controls for tone intensity and frequency, a timing system for control of ISI and stimulus duration, and a system for recording the response. The recording system consists of an electromagnet mounted below the cage, signal amplification, noise filtering, and display.

The three primary task parameters--tone intensity (1), interstimulus interval (2), and adaptation period--were evaluated using the new apparatus to determine which set of conditions produce the most consistent learning curves. Tone intensity was varied in Study 1 with ISI and adaptation period determined in Study 2. Using the information from Studies 1 and 2, a third study was conducted to determine if the task could be used as a repeated measure of learning ability on the same animals.

Study 1

Study 1 consisted of testing 4 groups of rats (N = 9/group) employing 4 tone intensities (db level) as indicated in Table 1, column A. Three animals from each group were tested on each of 3 test days. The sequence of testing was alternated each test day to avoid time-of-day effects (Table 1, column B). The tone frequency was consistent at 4 kHz and was presented for 100 ms. Each animal was given a 1-min adaptation period followed by 20 tone presentations with an ISI of 16 s.

TABLE 1. EXPERIMENTAL CONDITIONS FOR EACH OF THE 4 TEST GROUPS IN STUDY 1

A. Test conditions		B. Time sequence				
Tone intensity (db)*	Group number			Test day 1	Test day 2	Test day 3
96	1					
102	2	Start	0800	1**	4	3
110	3	Test	0830	2	1	4
118	4	Time	0900	3	2	1
			1000	4	3	2

*See Appendix for measurement methods.
**Group number

Study 2

Study 2 evaluated 4 new test groups to determine the effects of adaptation period and ISI (Table 2). The test sequence was the same as that used in Study 1 (Table 1, column B) with 3 animals from each group tested each test day. The tone intensity was set at 118 db (100 ms duration) since this intensity was shown in Study 1 to produce consistent learning curves (see Results).

TABLE 2. EXPERIMENTAL CONDITIONS FOR EACH OF THE 4 TEST GROUPS IN STUDY 2

		ISI (s) 16	ISI (s) 32
Adaptation period (min)	1	1	2
	3	3	4

Note: The number of each block is group number with N=9 for each group.

Study 3

Study 3 tested 12 naive rats repeatedly under the following conditions: tone intensity of 118 db (100-ms duration), ISI = 16 s, and an adaptation period of 1 min. There were 4 tests of the group with 3 days between each test.

RESULTS

The data generated in Study 1 for the 118 db group are illustrated in Figure 1. Employing linear curve fitting, we determined that the slopes generated by the first 6 responses of the 20 original were a clear index of learning ability. The results of Study 1 (tone intensity vs. response magnitude) are also presented in Figure 2, as the slopes of a linear model for the first 6 responses. The only slope significantly different from zero (linear regression: $P < .005$) was for the 118-db intensity setting. Therefore, the 118-db setting was used in the second study.

Study 2 was designed to determine if the adaptation period or ISI were dominant task parameters. The results are illustrated in Figure 3. A significant learning curve (linear regression; $P < .05$) was obtained for each test group (Table 2). The learning curves (slopes) were not significantly different across groups (Student-t).

Repeated testing of the same animals (Study 3) produced significant learning curves for the first 2 runs as judged by the slope generated by the first 6 responses of each linear regression ($P < .05$). The slopes of the learning curves are listed in Table 3. Runs 3 and 4 resulted in learning curves with increased variance. The data from runs 3 and 4 suggest that the subjects recall the stimuli.

TABLE 3. RESULTS, SLOPES OF LEARNING CURVES, OF REPEATED TESTING OF THE SAME ANIMALS ON 4 CONSECUTIVE DAYS

Run day	Slope ±standard error	P value (Linear regression)
1	-0.74 ± 0.07	<0.001
2	-0.33 ± 0.03	<0.001
3	0.15 ± 0.22	>0.4
4	-0.30 ± 0.19	>0.3

The consistency of the learning curves across test groups can be compared by using the groups from each study with identical test parameters: Study 1, group 4 (Table 1, column A); Study 2, group 1; and the first run from Study 3. All 3 of these groups were tested at 118 db, 1-min adaptation, and an ISI of 16 s. The learning curves of these groups are presented in Figure 4. The slopes of the 3 curves (first 6 responses) were not significantly different (Student-t).

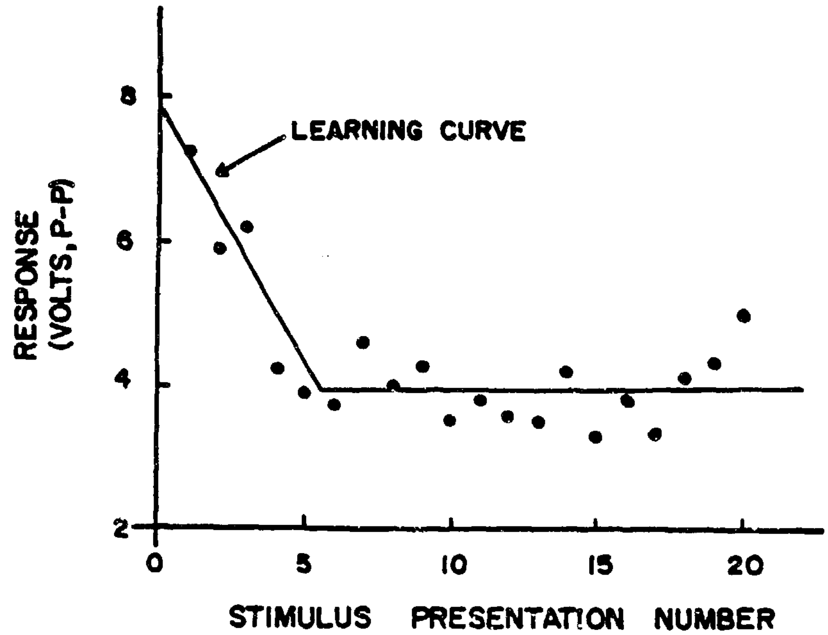

Figure 1. Startle response magnitude vs. the number of startle stimuli presented (ISI - 16 s). The slope of the learning curve, habituation to startle, was -0.73 with a linear correlation of 0.94.

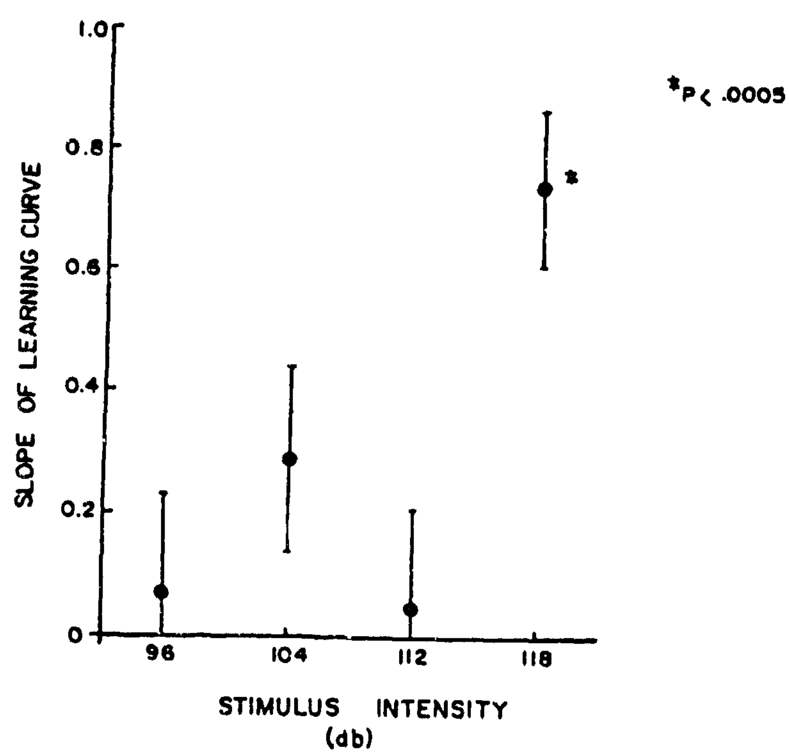

Figure 2. Slope of learning curves as a function of stimulus intensity. Mean ± standard deviation shown.

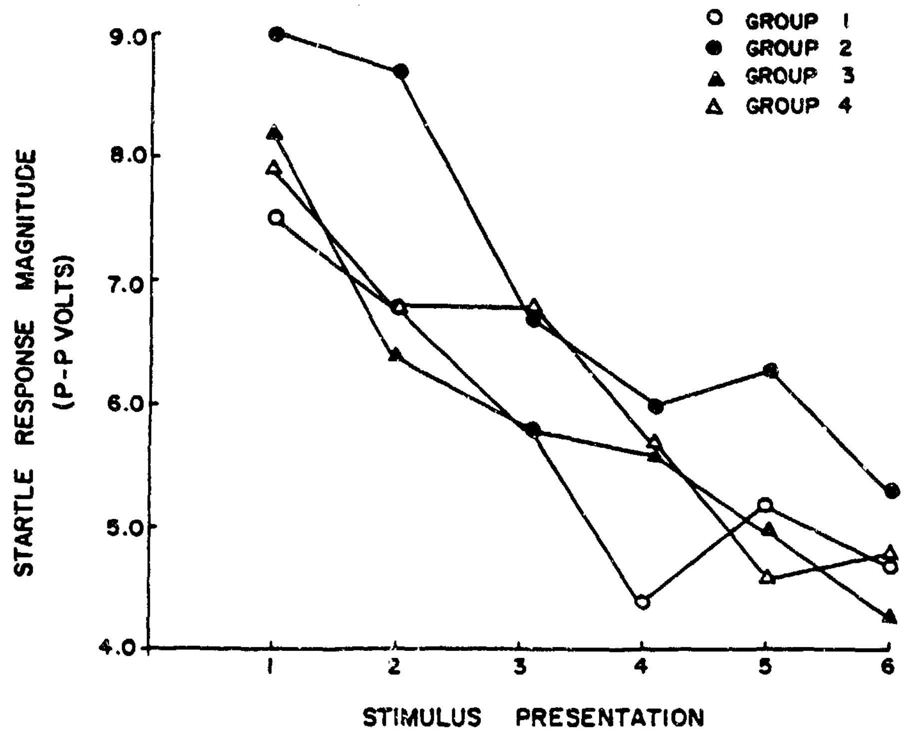

Figure 3. Startle response magnitude vs. number of stimuli presentations for 4 independent test groups. The experiment variables were adaptation period (AP) 1 and ISI. Group 1, conditions were a 1-min AP, 16-s ISI; Group 2 - 1-min AP, 32-s ISI; Group 3 - 3-min AP, 16-s ISI; and Group 4 - 3-min AP, 32-s ISI.

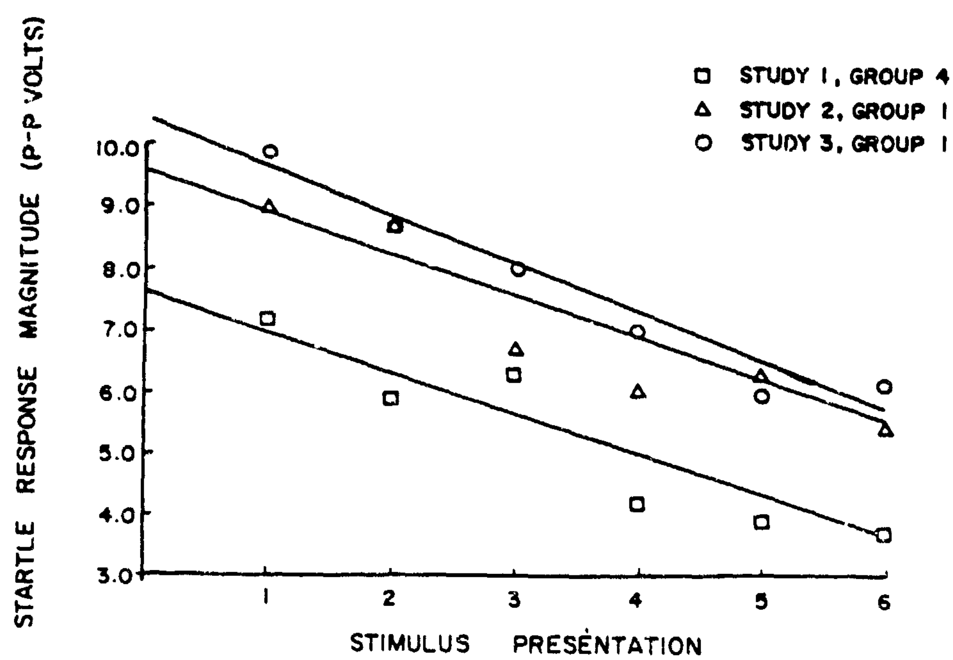

Figure 4. Startle response vs. number of stimulus presentations for 3 independent test runs, all employing the same experimental conditions.

DISCUSSION

The results clearly prove that the startle reflex can be used as a learning task. Learning can be proved using as few as 6 stimulus presentations. Of the test parameters evaluated, only tone intensity and repeated testing affected the learning curve. Changing the ISI or adaptation period had no significant effect on the learning curves over the range used (Table 2).

We thought that the startle learning task might be used as a repeated measure of learning ability. The data did not support this contention, but does suggest that testing the same group twice would result in consistent learning curves (Table 3). Therefore, the task could be used as a pre- and post-treatment measure of learning, if the treated group's data were compared to a control group's data. Repeated testing (more than twice) resulted in less pronounced learning curves with much greater subject-to-subject variance.

We have shown that the task produces consistent results across test groups. When test parameters are held constant, the results indicate consistent learning curves (Fig. 4). The task should prove a valuable addition to the rodent test battery; particularly when the test agent produces motor deficits. Employing the startle task should allow segregation of learning ability from mobility deficits.

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APPENDIX

STARTLE REFLEX APPARATUS

General

The startle reflex apparatus will be discussed in 3 major sections: (1) startle reflex monitoring system, (2) startle stimulus generator, and (3) stimulus control system.

Startle Reflex Monitoring System

The startle reflex monitoring system consists of a Plexiglas box resting on a speaker, an operational amplifier, and an oscilloscope. The holding box is made of Plexiglas with an open top. The box rests on another piece of Plexiglas which is supported by 4 rubber stoppers and physically attached by means of a fifth rubber stopper to the center of a 7.62-cm (3-in.) speaker cone. Movement of the holding box in the vertical plane causes a voltage to be induced within the speaker coil. This voltage is then fed into an operational amplifier. A 10-turn potentiometer has been built into the gain circuit so that a gain may be selected from unity to 10K. A memory oscilloscope is used to record the amplified signal. Calibration was done by measuring the voltages produced by dropping steel balls of various weights from the top of the holding box (Fig. A-1).

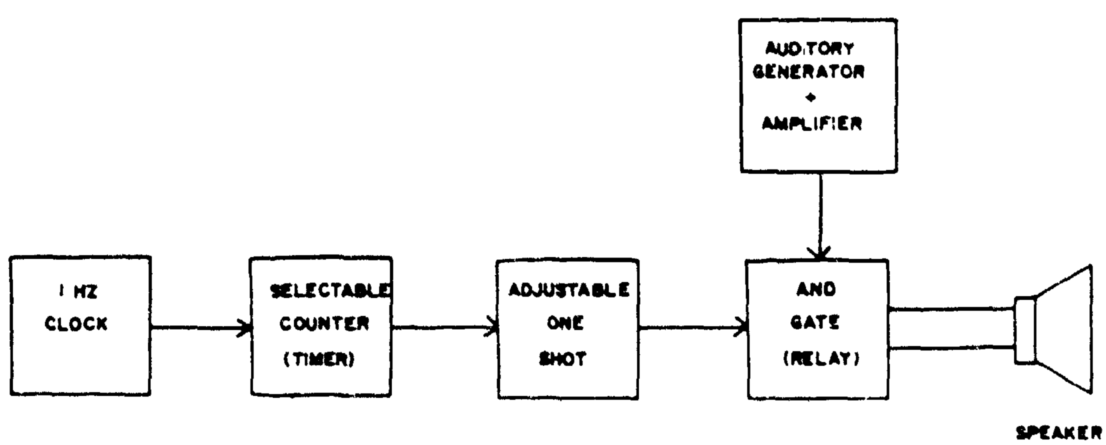

Figure A-1. Monitoring system.

Startle Stimulus Generator

The startle stimulus generator was obtained by means of feeding the output of a function generator set at 4 kHz (sine wave) to an audioamplifier. The output of the audioamplifier drives a 7.89-cm (3 1/2-in.) speaker, with the speaker placed in an acoustic chamber, the system was capable of producing 129 db (see Table A-1).

TABLE A-1. AUDIOAMPLIFIER OUTPUT VS. DIAL SETTING

Setting	db
10	129.1
9	125.7
8	122.8
7	118.3
6	108.4
5	104.5
4	99.6
3	94.0
2	89.1
1	below background
0	" "

Stimulus Control System

The schematic for the control system is illustrated in Figure A-2. A clock produces a 1-Hz signal which is sent to a binary counter. The counter will put out a pulse after a selectable delay from 1 to 32 s. The counter output pulse triggers a one shot with a selectable pulse duration. The one-shot pulse activates a relay, which connects the output of the audioamplifier to the speaker. The control system is capable of enabling the speaker at a selectable ISI (delay). The speaker was enabled for 100 msec for the studies.

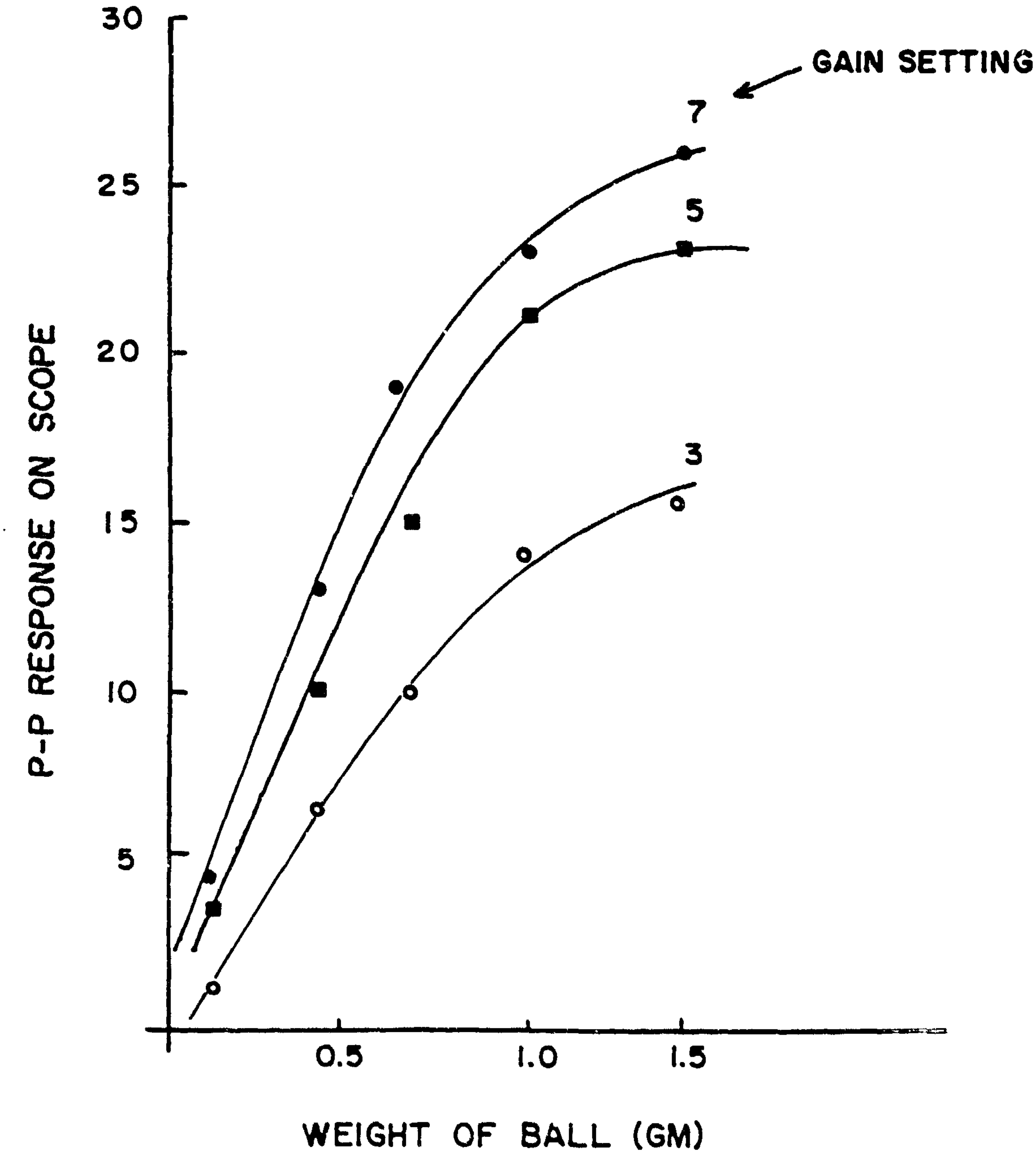

Figure A-2. Control system schematic.